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# Study of Anti-Inflammatory Activity *in vitro* of four Essential Oils from Gabon

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**Abstract** **Original Research Article**

The present study was designated for the evaluation of the anti-inflammatory activity of four essential oils (*Aucoumea klaineana* Pierre, *Canarium schweinfurthii* Engl., *Dacryodes buettneri* HJ Lam, *Dacryodes edulis* (G. Don) HJ Lam) derived from medicinal plants from Gabon using the *in vitro* model. The anti-inflammatory activity of essential oils was determined using the method of denaturing the proteins of egg albumin. Essential oil of *Aucoumea klaineana* ($IC_{50}$ = 1.11 ± 0.02 µg / mL) showed better thermal anti-denaturation efficacy of ovalbumin, followed by *Dacryodes buettneri* ($IC_{50}$ = 02.95 ± 0.69 µg / mL) and *Dacryodes edulis* ($IC_{50}$ = 09.32 ± 1.58 µg / mL). *Canarium schweinfurthii* essential oil ($IC_{50}$ = 13.51 ± 1.34 µg / mL) exhibited weak activity compared to other essential oils, however, its inhibitory action is more effective than Diclofenac sodium ($IC_{50}$ = 22.39 ± 1.04 µg / mL). This study supports the traditional use of these plants to treat certain disorders linked to inflammation.

**Keywords**: Anti-Inflammatory, essential oil, *Aucoumea klaineana*, *Canarium schweinfurthii*, *Dacryodes buettneri*, *Dacryodes edulis*.



## INTRODUCTION

Inflammation is the body's natural immune response to various aggressions which can be of physical, chemical, biological or infectious origin. Its current treatment is based on steroidal anti-inflammatory drugs (glucocorticoids) and nonsteroidal. These molecules, although being effective, most often have undesirable effects which can hinder their long-term use [1].

Since antiquity, medicinal plants (essential oils or extracts) have played an important role in the prevention of human health. They continue to provide humanity with new remedies. It is therefore important to explore medicinal plants for their safety, quality, toxicity and effectiveness. Researchers are very interested in essential oils in order to combine them with existing molecules to increase their effectiveness [2].

Our interest was focused on the study of four essential oils (*Aucoumea klaineana* Pierre, *Canarium schweinfurthii* Engl., *Dacryodes buettneri* HJ Lam, *Dacryodes edulis* (G. Don) HJ Lam) from medicinal plants widely used in traditional medicine in Gabon.

*Aucoumea klaineana* Pierre is a tree characteristic of the equatorial type forests, of secondary origin, its resin makes it possible to treat abscesses. The bark can be used as an antiseptic, astringent or antidiarrheal [3-5].

*Canarium schweinfurthii* Engl is a variety of large tree with compound leaves, with numerous predominant lateral veins. The resin is used in traditional medicine for the treatment of various diseases such as microbial wounds and infusions [6].

*Dacryodes buettneri* (Engl.) Guillaum, is a large tree, easily recognizable by its brilliant green leaves, with a reddish-white underside. Its resin is used to ripen abscesses. The thin platelets, which are removed from the bark, reduced to powder, are applied to burns (Walker and Sillans, 1961).

*Dacryodes edulis* is a tree that can exceed 50 m in height. Its pulverized bark is applied to wounds, it is emetic and pesticidal [3, 7].

The objective of our study is to verify in vitro whether the use of essential oils as anti-inflammatories is justified.

## MATERIALS AND METHODS

### Plant Materials

The resins of *Aucoumea klaineana*, *Canarium schweinfurthii*, *Dacryodes buettneri* and *Dacryodes edulis* were collected in December 2018 at the Sibang Arboretum (IPHAMETRA – Librevill – Gabon). Specimens were deposited at the National Herbarium of Gabon (IPHAMETRA). The essential oil was extracted from resin (500 g) by hydrodistillation in a clavenger-type apparatus for 4 h and was dried, after decantation, over anhydrous sodium sulphate.

### Evaluation of the anti-denaturing effect of proteins

In order to study the anti-inflammatory effect of essential oils, an in vitro evaluation test of the ability of these oils to prevent thermal denaturation of ovalbumin extracted from egg white by turbidimetry was used. In fact, heat causes the reversible change in the tertiary and even secondary conformation of proteins, which results in the reduction of their solubilities and the loss of their functions [8].

### Ovalbumin Extraction

Ovalbumin was extracted from chicken egg white according to the method described by Datta et *al.*, [9]. A fresh egg was broken and the yolk was separated from the white, the egg white was collected in a beaker put in an ice bath and mixed with 50 mL of phosphate buffer (0.1M, PH = 6.6) then homogenized under magnetic stirring for 5 min, then the homogenate obtained was centrifuged at 3000 rpm (4°C) for 5 min, the supernatant obtained was filtered through a strip of gauze to remove the precipitant. Finally, the filtrate obtained was partitioned into aliquots with a volume of 2 to 2.5 mL and then stored at -20°C.

### Measurement of the anti-denaturing activity of essential oils

Protein denaturation methods have been used [2, 10] with slight modifications. Briefly, 0.1 mL of albumin from fresh chicken eggs was mixed with 1.9 mL of phosphate buffered saline (PBS, pH 6.4) and 1 mL of varying concentrations essential oils so that final concentrations range from 18.87 to 0.10 mg / mL. A similar volume of distilled double water served as a negative control. Then, the mixtures were incubated at 37°C in an incubator (Ecocell, LSIS-B2V/EC55, Germany) for 20 min and then heated at 70°C for 5 min. After cooling, the absorbances were measured at 660 nm on the spectrophotometer (Evolution 60S, USA). Diclofenac sodium in the final concentrations was used as a reference drug and similarly treated for the determination of absorbance.

% Inhibition = $[(Abs_{sample}-Abs_{control}) / Abs_{control}]$ x 100, Abs = absorbance. The concentration of the extract for a 50% inhibition ($IC_{50}$) was determined by the dose response curve.

## STATISTICAL ANALYSIS

All data were measured average value of three replicates and standard error (±). Results were subjected to Microsoft excel 2013 and kaleidagraph version 4.0.

## RESULTS

To evaluate the anti-inflammatory activity *in vitro* of 4 essential oils (*Aucoumea klaineana* Pierre, *Canarium schweinfurthii* Engl., *Dacryodes buettneri* HJ Lam, *Dacryodes edulis* (G. Don) HJ Lam), we used the inhibition test of denaturing of ovalbumin extracted from chicken egg white. The 50% inhibitory concentrations of each essential oil are summarized in Table-1.

**Table-1: Effect of four essential and diclofenac sodium against protein denaturation**

| Plants / reference drug | $IC_{50}$ (µg/mL) | Equation | $R^2$ |
|---|---|---|---|
| *Aucoumea klaineana* | 1.11 ± 0.02 | y = 64.09x + 21.26 | $R^2$ = 0.99 |
| *Canarium schweinfurthii* | 13.51 ± 1.34 | y = 2.54x + 15.66 | $R^2$ = 0.98 |
| *Dacryodes buettneri* | 02.95 ± 0.69 | y = 6.68x + 30.23 | $R^2$ = 0.99 |
| *Dacryodes edulis* | 09.32 ± 1.58 | y = 3,68x + 15.67 | $R^2$ = 0.96 |
| Diclofenac sodium | 22.39 ± 1.04 | y = 1.95x +6.33 | $R^2$ = 0.96 |

From the 50% inhibition of ovalbumin denaturation, the concentrations of each essential oil were obtained. We find that all essential oils have the highest anti-inflammatory activities compared to Diclofenac sodium (reference anti-inflammatory).

Indeed, at the same concentrations of the different essential oils, *Aucoumea klaineana* ($IC_{50}$ = 1.11 ± 0.02 µg / mL) exhibited better anti-thermal denaturation efficacy of ovalbumin, followed by *Dacryodes buettneri* ($IC_{50}$ = 02.95 ± 0.69 µg / mL) and *Dacryodes edulis* ($IC_{50}$ = 09.32 ± 1.58 µg / mL). *Canarium schweinfurthii* essential oil ($IC_{50}$ = 13.51 ± 1.34 µg / mL) exhibited weak activity compared to other essential oils, however, its inhibitory action is more effective than Diclofenac sodium ($IC_{50}$ = 22.39 ± 1.04 µg / mL).

## DISCUSSION

Denaturation of proteins is a well-documented cause of inflammation. As part of the investigation into the mechanisms of anti-inflammation activity, the ability of essential oils from certain herbal plants to inhibit the denaturing of proteins was studied. Indeed, the essential oils tested (*Aucoumea klaineana* Pierre, *Canarium schweinfurthii* Engl., *Dacryodes buettneri* H. J. Lam, *Dacryodes edulis* (G. Don) H. J. Lam) have an inhibitory effect on thermal denaturation, thus the stabilizing power of ovalbumin. The stabilization of proteins by these natural substances probably involves polyphenols and their metabolites which act as modulators of the signaling pathways of inflammation [11, 12].

The conformation of a protein is linked to the secondary and tertiary structure; it is carried out via low energy bonds (hydrogen, electrostatic, hydrophobic bonds and disulfide bridges) therefore fragile. Denaturation results from a modification of the quaternary, tertiary and secondary structures without fragmentation of the peptide chain under the effect of various chemical (acid, base, detergent) or physical (heat, PH) agents [13]. The increase in temperature generates a thermal agitation of the atoms of the molecule, therefore the denaturation of proteins goes through ephemeral structures which can lead to a total unfolding of the molecule but it is also considered that denaturation can result from an increase in structure beyond the native form. The expansion similar to a statistical ball structure increases the stability of the molecules. This denaturation modifies the properties of proteins [13].

The protein denaturation inhibition test is used *in vitro* to an anti-inflammatory activity evaluation test. The denaturation of a protein causes the induction of the inflammatory reaction by the production of auto-antigens, important factors for developing chronic inflammation. This test is carried out in order to measure the inhibitory power of the thermal denaturation of protein by essential oils of Gabonese medicinal plants.

## CONCLUSION

Nowadays, a large number of medicinal plants have very important biological properties certainly linked to their therapeutic properties. During this study, we evaluated the anti-inflammatory activity *in vitro* of four essential oils from medicinal plants in Gabon. The study shows that the *in vitro* anti-inflammatory activity of essential oils has proven protein stabilization activity against thermal denaturation with efficiency comparable to that of the non-steroidal anti-inflammatory drug decofenac. These results remain preliminary and require further in-depth studies through anti-inflammatory activities in other systems *in vitro* (cellular and enzymatic) and *in vivo* (animal model) to better understand the molecular interactions of these compounds vis-à-vis of their targets.

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